

IN THE SPECIFICATION

Please amend the title as requested by the Examiner as follows:

**METHODS FOR ALTERING THE CLEAVAGE SPECIFICITY OF
A TYPE IIG RESTRICTION-ENDONUCLEASE A METHOD FOR
CREATING A CHIMERIC TYPE IIG RESTRICTION
ENDONUCLEASE AND DETERMINING ITS RESTRICTION
ENDONUCLEASE ACTIVITY**

Please amend the paragraph following the heading, "CROSS REFERENCE" to update the status of a priority application as requested by Examiner as follows:

This application is a continuation-in-part of U.S. patent application 10/150,028 filed May 17, 2002, abandoned, which is a divisional application of U.S. application 09/693,146 filed October 20, 2000, now U.S. Patent No. 6,413,758 issued July 2, 2002, each of which is hereby incorporated by reference in its entirety.

Please amend the figure legend for Figure 9 (added in the response dated November 17, 2007) as follows:

Figure 9 shows the sequence alignment of Gamma type methylase motifs from AccI, BanIII, BsuBI, CviBIII, Eco57I, HincII, PaeR7I, PstI, TaqI, TthHB8I, VspI, EcoRI, COMtase and HhaI (SEQ ID NOS:27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, respectively) from alignment of N6mA DNA Mtases and 9N4mC DNA Mtases as described in Figure 1c of Malone et al. *J. Mol. Biol.* 253:618-632 (1995).

Please amend the paragraph beginning on page 34, line 18 to insert two sequence identifiers as requested by the Examiner as well as to update the address of New England Biolabs:

The DNA recognition sequences for *BpmI* and *BsgI* are CTGGAG and GTGCAG, respectively. *BsgI* endonuclease is a Type IIG enzyme that shares 35.4% amino acid sequence identity to *BpmI*. A chimeric enzyme was constructed between *BpmI* and *BsgI*, in which the N-terminal coding sequence (catalytic domain plus methylase motifs I to III) was derived from *BpmI* and the C-terminal coding sequence (methylase motifs IV to X and the specificity domain) was derived from *BsgI*. The chimeric coding sequence was generated by a two-step PCR reaction. PCR primers were designed that can anneal to methylase motif IV on both *BpmI* and *BsgI* templates. The amino acid sequences in the fusion junction are shown below:

BpmI F D A I I G N P P Y (SEQ ID NO:41)

BsgI F D V I L G N P P Y (SEQ ID NO:42)

The forward primer P1 described in Example 5 and a new reverse primer P2' were used to amplify the N-terminal coding sequence from *BpmIRM* gene.

The new reverse mutagenic primer P2' has the following sequence:
5' ATAGGCTGGATTGCCTAATATTACATCAAAGCCACCATTTGC 3' (P2').
(SEQ ID NO:25)

PCR conditions were 94°C for 5 min, 1 cycle; 94°C for 30 sec, 55°C for 30 sec, 72°C for 2 min for 17-22 cycles; 72°C, 7 min for 1 cycle with 4 units of Vent® DNA polymerase.

The forward mutagenic primer in the fusion junction has the following sequence:

5' TTTGATGTAATATTAGGCAATCCACCCTATATAAGAATTC 3' (P3')
(SEQ ID NO:26)

Since the *BsgIRM* gene was cloned in pUC19, primer P3' and a pUC universal primer NEB #1221 was used to amplify the C-terminal *BsgI* coding sequence. PCR conditions were 94°C for 5 min, 1 cycle; 94°C for 30 sec, 55°C for 30 sec, 72°C for 2 min for 15-22 cycles; 72°C, 7 min for 1 cycle with 4 units of Vent® DNA polymerase. The PCR products were purified from a low-melting agarose gel and assembled by PCR using primers P1' and pUC universal primer #1221 (New England Biolabs, Inc., Ipswich Beverly, MA). The PCR conditions were 94°C for 5 min, 1 cycle; 94°C for 30 sec, 55°C for 30 sec, 72°C for 3 min 10 sec for 15 cycles; 72°C, 7 min for 1 cycle with 4 units of Vent® DNA polymerase. The PCR DNA fragment was cloned into pET21at and transformed into T7 expression host ER2566. *E. coli* host with pACYC-*BpmIM* or pACYC-*BsgIM* was also used for transformation. The fusion junction was confirmed by DNA sequencing.